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Glycosylation of human receptor guanylyl cyclase C

Najla Arshad* and Sandhya S Visweswariah

Address: Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore-560012, India

Email: Najla Arshad* - n_arshad@mrdg.iisc.ernet.in

* Corresponding author

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Background

Post translational modifications regulate several signalling pathways and glycosylation is emerging as a key player in this regulatory process. Glycosylation of cell surface receptors modulate the downstream signalling pathway and when altered causes a change in the normal physiology of the cell [1]. Guanylyl cyclase C (GC-C) belongs to a group of membrane bound receptors that produce the second messenger, cGMP [2]. It is a multidomain protein in which the extracellular domain (ECD) is N-glycosylated, resulting in the expression of two differentially glycosylated forms of GC-C (130 kDa and 145 kDa) [3]. Apart from the endogenous peptide ligands guanylin and uroguanylin, GC-C is activated by the heat stable enterotoxins (ST) produced by pathogenic *Escherichia coli* [2]. It is only the 145 kDa form which is activated by ST – a property attributed to the sugars present on it as both forms bind the ligand with comparable affinities as does the non-glycosylated ECD expressed in *E. coli* [4].

Infection with enteropathogenic *E. coli* hyperactivates GC-C resulting in diseases such as infant mortality and travellers' diarrhoea in adults. The glycosylation profile in the infant intestine changes with time and diet which perhaps leads to this differential response to ST in infants and adults [5]. Glycosylation is also tissue specific, which may result in differential regulation of GC-C present in extraintestinal tissues [6]. Altered glycosylation in cancer cells [7] may also influence GC-C activity. Thus, this study aims to understand the effect glycosylation has on GC-C function, which may in turn influence cGMP production.

Results

Treatment of cells expressing GC-C with wheat germ agglutinin resulted in inhibition of GC-C activity, while treatment with Concanavalin A did not. This differential response suggests that a specific lectin-sugar interaction prevents receptor activation.

In order to determine the sites of glycosylation responsible for receptor activation, a mutational approach was undertaken. The ten asparagine residues which are putative N-glycosylation sites in the ECD were mutated to alanine. All mutant receptors showed ST-mediated cGMP production in intact cells, but mutations at Asn345 and Asn402 showed a 40% reduction in ST-stimulatability. Western blot analysis of the single mutants showed differences in electrophoretic mobility between mutant and wild type receptors, due to loss of glycosylation.

Conclusion

Apart from activation, glycosylation also protects proteins from proteolytic cleavage. This is significant in the case of GC-C given its localization in the intestinal lumen, where proteolytic enzymes abound. The susceptibility of the N345 mutant to tryptic cleavage revealed that glycosylation may be of physiological importance in the intestinal milieu. Thus the varied roles of glycosylation in GC-C are emerging and the specificity of these roles at different glycosylation sites are being investigated.

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